Claims

1. Method of modeling and/or obtaining tissue or tissue-like structures comprising culturing an embryonic stem (ES) cell-derived first cell type in the presence of at least one embryonic second cell type; and allowing integration and alignment of said at least two cell types into tissue or tissue-like structures.

2. The method of claim 1, wherein the ES cell of said ES cell-derived first cell type comprises a selectable marker operably linked to a first cell type-specific regulatory sequence specific for said first cell type.

3. The method of claim 2, wherein said selectable marker confers resistance to puromycin.

4. The method of any one of claims 1 to 3, wherein said ES cell of said ES cell-derived first cell type comprises a reporter gene operably linked to a cell type-specific regulatory sequence specific for said first cell type.

5. The method of claim 4, wherein said cell type-specific regulatory sequence of the reporter gene is substantially the same as said first cell type-specific regulatory sequence of the marker gene.

6. The method of claim 5, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

7. The method of any one of claims 4 to 6, wherein said marker gene and said reporter gene are contained one the same recombinant nucleic acid molecule.

8. The method of claim 7, wherein said marker gene and said reporter gene are contained on the same cistron.

9. The method of any one of claims 1 to 8, wherein said first cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes,

chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

10. The method of any one of claims 1 to 9, wherein said first cell type are cardiomyocytes.

11. The method of claim 10, wherein said first cell type-specific regulatory sequence is atrial and/or ventricular specific.

12. The method of claim 10 or 11, wherein said at least one embryonic second cell type are fibroblasts or endothelial cells.

13. The method of any one of claims 1 to 12, further comprising culturing said at least two cell types in the presence of an embryonic or embryonic stem (ES) cell-derived third cell type.

14. The method of claim 13, wherein said third cell type are endothelial cells or fibroblasts.

15. A co-culture of cells as defined in any one of claims 1 to 14.

16. Tissue obtainable by the method of any one of claims 1 to 14.

17. A method of improving tissue repair and/organ function in a mammal comprising the steps of:

(a) introducing a cellular inoculum comprising a co-culture of cells of claim 15 in which differentiation has been initiated or tissue of claim 16 to at least a portion of the previously damaged area of the tissue; and

(b) allowing said introduced cellular inoculum to engraft in situ as viable cells or tissue situated within the previously damaged area of the tissue, wherein the engraftment results in improved tissue and/or organ function in said mammal.

18. A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising the steps of:

(a) culturing undifferentiated mammalian embryonic stem (ES) cells comprising a resistance gene and a reporter gene under the control of the same cardiac-specific promoter in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes;

(b) isolating said differentiated cardiomyocytes and/or eliminating non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types from said cardiomyocytes in the course of differentiation;

(c) subsequently co-transplanting said cardiomyocytes with embryonic or ES cell-derived fibroblasts to at least a portion of the previously infarcted area of the heart tissue; and

(d) allowing said introduced cellular inoculum to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

19. The method of claim 18, wherein said resistance gene and said reporter gene are contained in a bicistronic vector and separated by an IRES.

20. The method of claim 19, wherein said resistance gene confers resistance to puromycin, said marker is EGFP and said promoter is the cardiac αMHC promoter.

21. Method of modeling and/or obtaining tissue or tissue-like structures comprising the following steps:

(a) transfecting one or more multi- or pluripotent cells with recombinant nucleic acid molecules comprising a first and a second cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said second cell type is different from said first cell type;

(b) culturing the cells under conditions allowing differentiation of the cells; and

(c) isolating cells of at least two differentiated cell types and/or eliminating non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types from cell types of interest that activate the selectable marker in the course of differentiation.

22. The method of claim 21, further comprising transfecting said one or more cells with recombinant nucleic acid molecules comprising at least one further cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said at least one further cell type is different from said first and second cell type.

23. The method of claim 21 or 22, wherein said cells are embryonic stem (ES) or embryonic germ (EG) cells.

24. The method of any one of claims 21 to 23, wherein said recombinant nucleic acid molecules are comprised in the same vector or different vectors.

25. The method of any of claims 21 to 24, wherein said cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes, chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

26. The method of claim 7 or 8, wherein said promoter is selected from the group consisting of αMHC, MLC2V, catherin, Tie-2 and collagen promoter.

27. The method of any one of claims 21 to 26, wherein said one or more recombinant nucleic acid molecules are transfected concomitantly or subsequently into said one or more cells.

28. The method of any one of claims 21 to 26, wherein at least two different cells or clones thereof are transfected and selected, wherein said at least two different cells or cell clones contain recombinant nucleic acid molecules with different cell type specific regulatory sequences.

29. The method of claim 28, wherein said at least two different cells or cell clones are mixed at the initial stage of differentiation in order to allow formation of cell aggregates.

30. The method of claim 29, wherein said cell aggregates are chimeric embryoid bodies (EBs).

31. The method of any one of claims 21 to 30, wherein one of said cells or cell clones thereof is transfected and selected, wherein said cell or cell clone contains recombinant nucleic acid molecules with at least two different cell type-specific regulatory sequences.

32. The method of any one of claims 21 to 31, wherein at least two of said selectable marker operably linked to said different cell type specific regulatory sequences are identical.

33. The method of any one of claims 21 to 32, wherein at least one of said selectable marker is operably linked to said different cell type-specific regulatory sequences confers resistance to puromycin, bleomycin, hygromycin, methothrexate, or neomycin.

34. The method of any one of claims 21 to 33, wherein one or more of said recombinant nucleic acid molecules further comprise a reporter operably linked to said cell type-specific sequence.

35. The method of claim 34, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

36. The method of claim 35, wherein EYFP (yellow), ECFP (blue) and/or hcRFP (red) are operably linked to different cell type-specific sequences.

37. The method of any one of claims 34 to 36, wherein said selectable marker and said reporter are expressed from a bicistronic vector.

38. The method of claim 37, further comprising one or more internal ribosomal entry sites (IRES), wherein said IRES separates said selectable marker and said reporter.

39. The method of any one of claims 21 to 38, further comprising allowing self-assembly of the different cell types.

40. The method of any of claims 1 to 14 or 21 to 39, further comprising analysing the physiological and/or developmental status of the cells or cell aggregate.

41. The method of claim 40, wherein the status is analyzed by monitoring the differentiation of electrical activity of the cells on an array.

42. The method of claim 41, wherein said status is analyzed by recording the extracellular field potentials with a microelectrode array (MEA).

43. A cell or cells obtainable by the method of any one of claims 21 to 42, wherein said cell or cells are capable of differentiating into at least two cell types.

44. A cell aggregate of at least two different cell types obtainable by the method of any one of claims 21 to 42.

45. A tissue obtainable by the method of any one of claims 1 to 42 or comprising cells of claim 43 or a cell aggregate of claim 44.

46. An organ comprising cells of claim 43, a cell aggregate of claim 44 or tissue of claim 45.

47. An implant or transplant comprising cells of claim 43, a cell aggregate of claim 44, a tissue of claim 45, or an organ of claim 46.

48. A composition of matter comprising recombinant nucleic acid molecules as defined in any one of claims 21 to 42, cells of claim 43, a cell aggregate of claim 44, or a tissue of claim 45.

49. Use of the method of any one of claims 1 to 14 or 21 to 42 for analyzing early steps of tissue formation during embryonic development or the influence of factors and compounds on this process.

50. A method of treatment of damaged tissue or organs in a subject comprising implanting or transplanting to the subject in need thereof cells of claim 43, a cell aggregate of claim 44, a tissue of claim 45 or an organ of claim 46.

51. A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising the steps of:

(a) transfecting mammalian embryonic stem (ES) cells with a recombinant nucleic acid molecule comprising a resistance gene under the control of cardiac, fibroblast and/or endothelium-specific regulatory sequences, and optionally comprising one or more reporters under the same specific regulatory sequences;

(b) culturing said ES cells in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes, fibroblasts and/or endothelial cells;

(c) eliminating from said differentiated cardiomyocytes, fibroblasts and/or endothelial cells non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types; optionally

(d) allowing aligning and integration of said differentiating cardiomyocytes, fibroblasts and/or endothelial cells into cardiac-like tissue;

(e) subsequently co-transplanting said cardiomyocytes, fibroblasts and/or endothelial cells or said tissue to at least a portion of the previously infarcted area of the heart tissue; and

(f) allowing said introduced cells or tissue to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

52. The method of claim 51, wherein said cardiomyocytes, fibroblasts and/or endothelial cells are derived from the same ES cell.

53. The method of claim 51, wherein said cardiomyocytes, fibroblasts and/or endothelial cells are derived from different ES cells.

54. The method of any of claims 51 to 53, wherein said cardiac-specific regulatory sequence is selected from promoters of αMHC, MLC22v, MLC1a, MLC2a and βMHC, said fibroblast-specific regulatory sequence is selected from promoters of Tie2, Tie1 and Catherin, and said endothelium-specific regulatory sequence is selected from promoters of collagen I promoters.

55. The method of any of claims 51 to 54, wherein said reporter for said cardiomyocytes, fibroblasts and/or endothelial cells is independently selected from the enhanced green fluorescent proteins ECFP (blue), EYFP (yellow) and hcRFP (red).

56. The method of any of claims 51 to 55, wherein said resistance gene and said reporter are separated by an internal ribosomal entry site (IRES).

57. A vector or a composition of vectors comprising the recombinant nucleic acid molecules as defined in any one of claims 51 to 56.

58. A cell or a plurality of cells comprising the vector or the composition of vectors of claim 57.

59. An array comprising a solid support and attached thereto or suspended thereon cells of claim 43, a cell aggregate of claim 44, or a tissue of claim 45.

60. The array of claim 59, which is a microelectrode array (MEA).

61. An apparatus for analyzing the array of claim 59 or 60.

62. A method for obtaining and/or profiling a test substance capable of influencing cell development and/or tissue structure formation comprising the steps:

(a) contacting a test sample comprising cells of claim 43, a cell aggregate of claim 44, a tissue of claim 45, an organ of claim 46 or an array of claim 59 or 60 with a test substance; and

(b) determining a phenotypic response in said test sample compared to a control sample, wherein a change in the phenotypic response in said test sample

compared to the control sample is an indication that said test substance has an effect on cell development and/or tissue structure formation.

63. The method of claim 62, wherein said test sample is contacted with said test substance prior to, during or after said cell or cell aggregate passed through the method of any one of claims 1 to 14 or 21 to 42.

64. The method of claim 62 or 63, wherein said contacting step further includes contacting said test sample with at least one second test substance in the presence of said first test substance.

65. The method of any one of claims 62 to 64, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

66. The method of claim 65, wherein said collection of test substances has a diversity of about 10^3 to about 10^5.

67. The method of claim 66, wherein the diversity of said collection of test substances is successively reduced.

68. The method of any one of claims 61 to 67, which is performed on an array as defined in claim 59 or 60.

69. The method of any one of claims 61 to 68, wherein the phenotypic response comprises electrophysiological properties during the ongoing differentiation process.

70. The method of any one of claims 1 to 14, 21 to 42 or 62 to 69, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

71. The method of any one of claims 1 to 14, 21 to 42 or 62 to 70, wherein a compound known to activate or inhibit differentiation process and/or tissue structure formation is added to the culture medium.

72. The method of any one of claims 1 to 14, 21 to 42 or 62 to 71, wherein said one or more cells or tissue are contained in a container.

73. The method of any one of claims 1 to 14, 21 to 42 or 62 to 72, comprising taking 3 or more measurements, optionally at different positions within the container.

74 The method of any one of claims 72 or 73, wherein said container is a well in a microtiter plate.

75. The method of claim 74, wherein said microtiter plate is a 24-, 96-, 384- or 1586- well plate.

76. A method of manufacturing a drug comprising the steps of any one of claims 62 to 75.

77. A method of manufacturing an agent which supports wound healing and/or healing of damaged tissue comprising the steps of any one of claims 62 to 76.

78. The method of claim 76 or 77, further comprising modifying said substance to alter, eliminate and/or derivatize a portion thereof suspected causing toxicity, increasing bioavailability, solubility and/or half-life.

79. The method of any one of claims 76 to 78, further comprising mixing the substance isolated or modified with a pharmaceutically acceptable carrier.

80. A kit or composition useful for conducting a method of any one of claims 1 to 14, 21 to 42, 50 to 56 or 62 to 79, containing the vector or the composition of vectors of claim 57, a multi- or pluripotent cell, and optionally culture medium, recombinant nucleic acid molecules, or standard compounds.

81. Use of cells of claim 43, a cell aggregate of claim 44, a tissue of claim 45 or an organ of claim 46, the implant or transplant of claim 47, the vector or the composition of vectors of claim 57, the composition of claim 48, an array of claim 59 or 60 or an apparatus of claim 61 in drug discovery or pharmacokinetic or pharmacological profiling.